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# Monoclonal anti-TNF $\alpha$ Antibody as a Probe of Pathogenesis and Therapy of Rheumatoid Disease

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## THE BURDEN OF RHEUMATOID DISEASE AND LIMITATIONS OF CURRENT THERAPY

Rheumatoid arthritis (RA) is a chronic disabling and painful disease of multiple joints which is prevalent in all parts of the world. Population studies suggest that around 1% of the population is affected, the disease being three times commoner amongst women than men. The peak incidence is in middle age but no age is exempt and it afflicts all age groups including children and the elderly. Although the disease spectrum is broad, ranging from mild to severe, the disabling effects are cumulative and, in the severe group, associated with premature death (Erhardt et al. 1989, Pincus & Callahan 1993).

Current management of RA is based around a multidisciplinary team with drug therapy playing a key role in alleviating symptoms, maintaining mobility and slowing the progression of joint damage. However, a great majority of patients require frequent changes in drug therapy due to loss of efficacy and/or to toxicity and the lack of significant control of disease becomes all too self-evident in the second decade from onset. Surgical treatment, including multiple joint prostheses, provides respite but highlights the failure of medical treatment. The economic, social and personal cost of RA is clearly unacceptable and motivates the search for better and more effective therapeutic and preventative measures.

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EXHIBIT

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# NEW THERAPIES BASED ON TARGETING DISEASE MECHANISMS

## Initiating factors and cellular targets

Attempts at discovery of effective novel cures for RA are based on the premise that knowledge of the causal factors and pathogenesis of RA has advanced significantly enough to construct hypotheses which define cellular and/or molecular targets. The etiology of RA is largely unknown, although population and family studies, together with observed concordance rates of disease in monozygotic twins, indicate that multiple germline genes are implicated with the genetic contribution to RA of 15% (Silman et al. 1993); however, a recent re-analysis has suggested that genetic factors could account for up to 60% of susceptibility (Macgregor & Silman 1994). The polymorphic HLA-DR  $\beta$  chain genes (some DR4 subtypes and DR1), encoding the 'shared epitope' pentapeptide sequence QRRRA, appears to be the main identified susceptibility gene (Gregersen et al. 1987). However, in the majority of patients a major role is attributed to non-genetic factors, and it is suggested that environmental factors, sex hormones and function of the hypothalamic-pituitary-adrenal axis, play a part (Reviewed in Silman & Hochberg 1993, Masi 1994, Chikanza et al. 1992). The association of RA with a HLA-DR molecule, which is involved in antigen binding and presentation to T cells, the presence in rheumatoid joints of activated memory T cells, and of autoantibodies in serum of patients, has suggested an important role for an immune response in initiating and maintaining RA. However, no convincing evidence for a rheumatoid specific antigen has been forthcoming, although antigens such as collagen II, abundant in cartilage, are possible candidates, and do elicit an antibody response in a majority of DR4 positive RA patients (Ronnellid et al. 1994). Indeed we have documented a single RA patient in whom collagen II specific T-cell clones were repeatedly cloned from joints over a period of several years (Londei et al. 1989), but, in general, we can only demonstrate T-cell reactivity to collagen II antigens in a minority of RA patients. Alternatively, analysis of T-cell receptors in the joints in some studies has pointed to a restriction and selection bias which could suggest the involvement of superantigens (Paliard et al. 1991).

Since the involvement of T cells is central to the immunological paradigm, a great deal of attention has been paid to characterizing the prominent T-cell infiltrate in RA joints. The results of these investigations have led to the general conclusion that CD4<sup>+</sup> cells of the memory phenotype which bear activation markers are predominant (Panayi et al. 1992). Although there is a lack of consensus on the level of T cell cytokine expression, with the majority of investigators only finding low, but detectable, levels of interleukin-2 (IL-2) and interferon  $\gamma$  (IFN $\gamma$ ) at protein level (Firestein & Zvaifler 1990, Buchan et al. 1988a), there is an emerging consensus that there is a deficiency of IL-4 secretion in established disease (Moissec et al. 1990, Simon et al. 1994). However, at mRNA level IFN $\gamma$

is consistently detected a predominance of Th compatible with a T  $\alpha$

These concepts of th approaches, summarizing sex hormones of ic-pituitary-adrenal ax which effectively 'block' by a putative R that collagen II is an immunodominant or c temic administration o strations that with sub are converted into ant

## Targeting

### Target

Sex hormones

Hypothalamic-pituitary-adrenal axis  
HLA DR4/I

Antigen

Superantigen

Th1 cells

T cells

Gut associated lymphoid system

## MECHANISMS

based on the premise that RA has advanced similar and/or molecular homology and family resemblance in monozygotic twins. The genetic contribution to the disease has been re-analysed and has suggested susceptibility (Macgregor) genes (some DR4) and peptide sequence (Gregersen et al.). The disease is attributed to non-genetic factors, sex hormones and part of the association of RA with the presentation of emory T cells, and of an important role for an immune response, no convincing evidence, although antigens identified, and do elicit responses in patients (Ronneld et al.) in whom collagen II is a period of several months. Alternatively, analysis of a restriction and superantigens (Paliard et al.) immunological paradigm, a prominent T-cell infiltrate led to the general conclusion which bears activation. There is a lack of consensus among investigators (IL-2) and interferon  $\gamma$  (et al. 1988a), there is secretion in established disease, at mRNA level IFN $\gamma$

is consistently detectable (Buchan et al. 1988a, Cohen et al. in press), suggesting a predominance of Th1 over Th2 subsets of T cells (Mossman & Coffman 1989), compatible with a T cell driven chronic inflammatory disease.

These concepts of the etiopathogenesis of RA have suggested many therapeutic approaches, summarized in Table I. These range from proposals that manipulating sex hormones or supplementing corticosteroids to restore the hypothalamic-pituitary-adrenal axis may be beneficial, to seeking synthetic designer peptides which effectively 'block' the HLA antigen binding site thus preventing its occupation by a putative RA specific autoantigen. For those investigators convinced that collagen II is an important autoantigen, attempts are being made to define immunodominant or cryptic epitopes that might induce tolerance either by systemic administration or via the oral route (Trentham et al. 1993). Recent demonstrations that with substitutions of one or two amino acids, immunogenic peptides are converted into antagonists, which result in immunological unresponsiveness

TABLE I  
Targeting initiating factors and the T cell response in RA

Target	Observation	Possible therapy
Sex hormones	Female preponderance Oral contraceptives protective	Anti-oestrogens, male sex hormones Progesterones
Hypothalamic-pituitary-adrenal axis	Disturbed, poor corticosteroid response to surgical stress	Early use of corticosteroids
HLA DR4/I	Association of 'shared epitope' with severe RA	Peptide blockade
Antigen	B and T cell collagen II reactivity	Induce tolerance by altered peptide ligands or soluble immunodominant peptides
Superantigen	Restricted TcR usage with CDR3 diversity	Induce tolerance with superantigens
Th1 cells	Predominant Th1 cell in joints is pro-inflammatory; IL-4, IL-12 anti-inflammatory and deviate to protective Th2 response	IL-4 (IL-10) therapy
T cells	Activated, CD4 <sup>+</sup> memory cells enriched in synovium	IL-2 diphtheria toxin conjugate and monoclonal antibody therapy, e.g., anti-IL-7, anti-CDW52, anti-CD4, anti-CD5
Gut associated lymphoid system	Oral collagen II induces bystander suppression via TGF- $\beta$ producing cells trafficking to joints	Oral collagen feeding



as promising therapies has been made in trials, and interactions of biological functions related to, for example, cytokines and leukocytes might adhere to this example. At

An appealing alternative therapeutic approach is based on exploiting the production of naturally occurring inhibitors of cytokines produced in RA joints. RA inflammation is characterized by enhanced, but insufficient, production of interleukin-1 receptor antagonist (IL-1ra) and soluble TNF receptors, which are the specific inhibitors of IL-1 and TNF $\alpha$  respectively (Deleuran et al. 1992a, Cope

et al. 1992), and of IL-10 (Katsikis et al. 1994), which inhibits synthesis of IL-1 and TNF $\alpha$ . Augmenting inhibitor production *in situ* or supplementing inhibitor by injecting recombinant soluble receptors, usually as multimeric-immunoglobulin fusion proteins, in order to increase affinity and biological half-life *in vivo*, provide further scope for anti-cytokine drug development.

#### PIVOTAL ROLE OF TNF $\alpha$

Our hypothesis that TNF $\alpha$  is a potential target for therapeutic intervention in RA was derived from experiments performed *in vitro* on RA synovial tissue. These studies indicated that dissociated synovial tissue mononuclear cells (MNCs) from RA patients were highly activated, and if placed in culture would produce cytokines spontaneously for 5–6 days without exogenous stimulation. The cytokines which are produced in abundance include IL-1 $\alpha$  and IL-1 $\beta$  (Buchan et al. 1988b), TNF $\alpha$  (Brennan et al. 1989), IL-6 (Hirano et al. 1988), IL-8 (Brennan et al. 1990b), TGF $\beta$  (Brennan et al. 1990a), GM-CSF (Haworth et al. 1991) and, as shown more recently, IL-10 (Katsikis et al. 1994). As IL-1 and TNF $\alpha$  have pro-inflammatory potential implicated in the pathogenesis of RA, we focused on the regulation of these cytokines.

Using a polyclonal anti-TNF antibody to block TNF $\alpha$  activity in the RA synovial cell cultures, we observed that this also resulted in the inhibition of IL-1 production (Brennan et al. 1989). The result suggested the presence of a 'cytokine cascade' within the synovium, with the prediction that blockade of a pivotal cytokine in this network would subsequently lead to blockade of other cytokines 'downstream'. This indeed was found to be the case in that the production of other pro-inflammatory cytokines including GM-CSF (Haworth et al. 1991), IL-6 and IL-8 (Butler et al. submitted) was also modulated by TNF $\alpha$  blockade. The biological effect of TNF $\alpha$  is enhanced further by our observation that both p55 and p75 surface TNF receptors are upregulated on RA synovial tissue cells (Brennan et al. 1991, Deleuran et al. 1992b) (Fig. 1). Indeed, CD68 positive macrophages at the cartilage pannus junction (Chu et al. 1992) also express abundant p55 and p75 TNF-R, indicating the potential of these cells to respond in an autocrine manner. The biological activity of TNF $\alpha$  is regulated to some degree, however, by the production of its native inhibitors soluble p55 and p75 TNF-R. These soluble TNF-R are also found in elevated amounts in RA plasma and in particular, in synovial fluid where their presence in excess neutralizes TNF $\alpha$  activity (Cope et al. 1992). The production of sTNF-R is insufficient however, as bioactive TNF $\alpha$  was found in all RA synovial MNC cultures tested.

A second immunoregulatory molecule, IL-10, is also abundantly produced by RA synovial MNC cultures (Katsikis et al. 1994). Endogenous IL-10 was found to be functional as its neutralization enhanced TNF $\alpha$  and IL-1 production 2–3 fold (Katsikis et al. 1994), indicating that as in the production of other cytokine

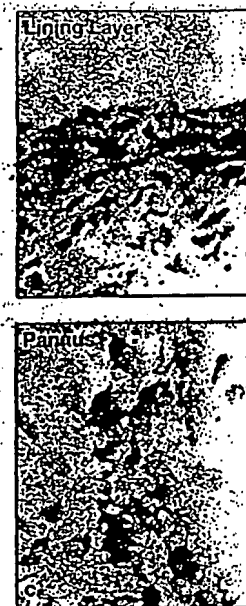


Figure 1. TNF $\alpha$  and p55 TNF-R at the cartilage-pannus junction in a (a) and TNF-R (b); similar cartilage-pannus junction.

inhibitors including sTNF-R (1992a) homeostatic mechanisms in insufficient quantities, biological changes continue.

#### EVIDENCE FOR THE

Murine collagen-induced arthritis (CIA) for human rheumatoid arthritis (RA) similarities between the two diseases include pannus formation and joint destruction. In addition, susceptibility to CIA is linked to MHC class II genes, and a similar association in both diseases is the M1 gene (1989).

The CIA model has been used to study the pathogenesis of human disease and

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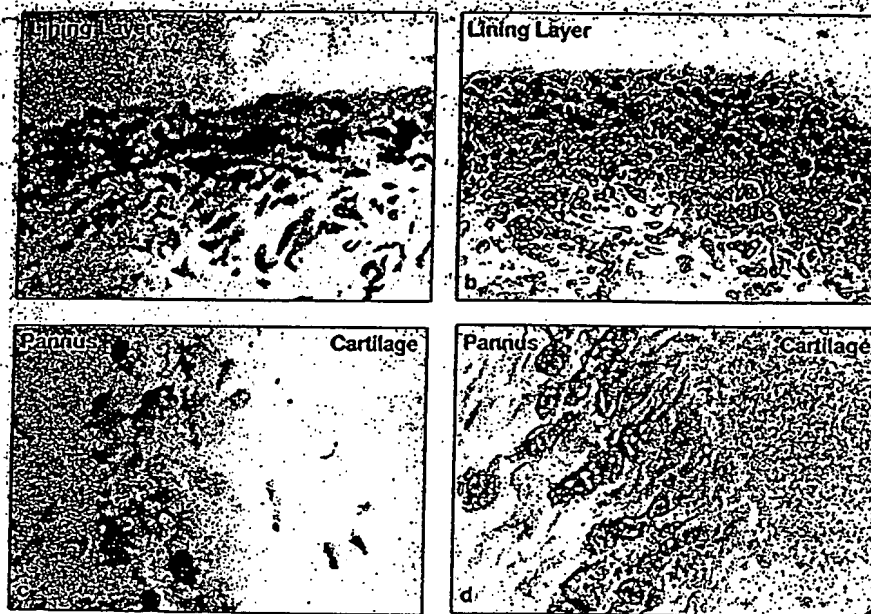


Figure 1. TNF $\alpha$  and p55 TNF-R demonstrated by immunohistology of synovium and cartilage-pannus junction in a RA joint. Majority of cells in synovial layer co-stain for TNF $\alpha$  (a) and TNF-R (b); similar co-localization of TNF $\alpha$  (c) and TNF-R (d) is observed at cartilage-pannus junction.

inhibitors including sTNF-R (Cope et al. 1992) and the IL-1ra (Deleuran et al. 1992a) homeostatic mechanisms are present in inflammatory environments, albeit in insufficient quantities, since cytokine production and the consequential pathological changes continue to occur.

#### EVIDENCE FOR THE IMPORTANCE OF TNF $\alpha$ FROM ANIMAL MODELS

Murine collagen-induced arthritis (CIA) has been extensively studied as a model for human rheumatoid arthritis (RA), principally because of the pathological similarities between the two diseases, including similar patterns of synovitis, pannus formation and erosion of cartilage and bone (Stuart et al. 1984). In addition, susceptibility to both human RA and murine CIA is linked to specific MHC class II genes, suggesting that an important step in the development of both diseases is the MHC-restricted activation of CD4<sup>+</sup> T cells (Holmdahl et al. 1989).

The CIA model has been used to elucidate pathogenic mechanisms of relevance to human disease and to identify potential targets for therapeutic intervention.

One of the methods used in such studies involves the administration of potentially pathological cytokines to naïve mice or to mice previously immunized with type II collagen in order to demonstrate altered expression of disease. For example, the local administration, in recombinant form, of IFN- $\gamma$ , IL-1 $\beta$ , or TNF to type II collagen-immunized mice has been shown to result in accelerated onset and increased severity of CIA, suggesting that these cytokines are involved in the induction of arthritis (Mauritz et al. 1988, Hom et al. 1988a, Thorbecke et al. 1992, Cooper et al. 1992, Brahn et al. 1992). None of the cytokines, however, was capable of initiating arthritis when injected into unimmunized mice, indicating that they could not, on their own, elicit a sustained inflammatory response. However, the experimental approach adopted in these studies has a number of limitations, including the fact that cytokines generally have extremely short half-lives *in vivo*.

An alternative approach would be to use transgenic mice to study the pathological effects arising from the sustained over-expression of cytokine transgenes. This approach powerfully demonstrated the arthritogenic potential of over-expression of TNF $\alpha$  in the joints of 3'-modified human TNFh-transgenic mice, which spontaneously develop a chronic arthritis that is prevented by anti-TNF treatment (Keffler et al. 1991). Our histopathological studies on the Kollis hTNF-transgenic mice show that the joints of these mice exhibit similar inflammatory lesions to those found in human RA, including proliferative synovitis and pannus formation. Most importantly, necrotic chondrocytes are observed in the joints of hTNF-transgenic mice as well as severe focal erosions of sub-chondral bone (Fig. 2). These findings clearly show that the dysregulated expression of TNF is alone capable of inducing many of the pathological changes that are seen both in human RA and murine CIA, including full-blown destruction of cartilage and bone.

An alternative to the study of cytokines that contribute to the pathogenesis of arthritis is to treat collagen-arthritic mice or rats with specific anti-cytokine agents in order to show a reduction in disease severity. Following the identification of pro-inflammatory cytokines, in particular TNF and IL-1, in the joints of RA patients (Chu et al. 1991, Chu et al. 1992), we and others set out to address the question of whether the neutralization of such cytokines would lead to amelioration of autoimmune arthritis. A number of studies focused on the effect of anti-TNF treatment in CIA, partly because of data accumulated in our laboratories suggesting that, in RA at least, TNF was playing an important role in the induction of other pro-inflammatory cytokines. In CIA, two studies showed that monoclonal or polyclonal anti-TNF antibodies, administered before the onset of clinical arthritis, protected against the subsequent development of disease (Thorbecke et al. 1992, Piguet et al. 1992). Similarly, soluble TNF receptors, which are thought to serve as endogenous regulators of TNF activity, also protected against CIA when given during the pre-arthritic period (Piguet et al. 1992).

Our own research has focused largely on the effects of anti-TNF treatment in

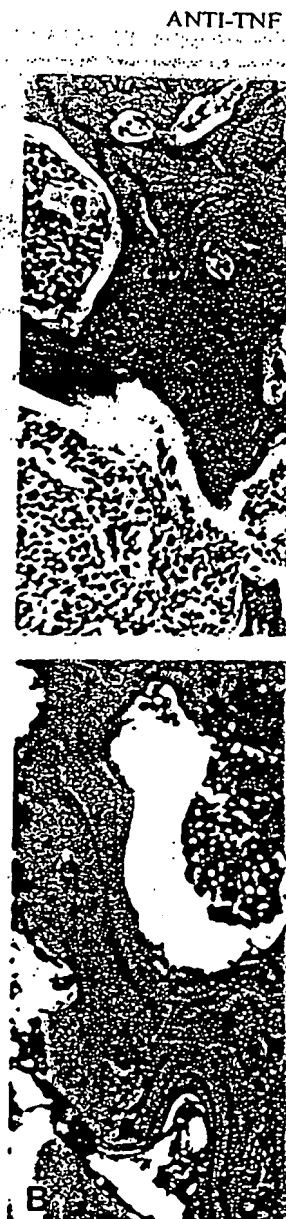


Figure 2. A: Light micrograph from a hTNF-transgenic mouse joint showing pannus and loss of chondrocytes. B: Light micrograph from a hTNF-transgenic littermate with chondrocyte loss.



administration of potentially self-immunized with type of disease. For example, IL-1 $\beta$ , or TNF to type in accelerated onset and cytokines are involved in the 1988a, Thorbecke et al. cytokines, however, was immunized mice, indicating inflammatory response. However, has a number of limitations extremely short half-lives

to study the pathologic cytokine transgenes. This potential of over-expression transgenic mice, which spontaneously TNF treatment Kollias hTNF-transgenic inflammatory lesions to arthritis and pannus formation in the joints of hTNF-transgenic chondral bone (Fig. 2). Expression of TNF is alone what are seen both in human of cartilage and bone. Due to the pathogenesis of the specific anti-cytokine following the identification of IL-1, in the joints of RA, we set out to address the question would lead to ameliorated on the effect of anti-TNF in our laboratories. The important role in the induction studies showed that monotherapy before the onset of clinical disease (Thorbecke and JF receptors, which are also, also protected against et al. 1992).

of anti-TNF treatment in



Figure 2. A: Light micrograph showing the cartilage-bone-pannus region of a knee joint from a hTNF-transgenic mouse. Note the focal erosion of sub-chondral bone by cellular pannus and loss of chondrocytes in cartilage. B: Normal joint and surfaces from non-transgenic littermate with chondrocyte-rich cartilage and normal bone.

established CIA, because of the greater significance of such studies in terms of human therapy. We were able to show that a hamster IgG1 anti-TNF monoclonal antibody (mAb) (TN3-19.12) administered over a 10-day period, starting immediately after the onset of clinically-detectable arthritis, caused a reduction in clinical score and suppressed paw-swelling, without altering circulating levels of anti-type II collagen IgG (Williams et al. 1992). In addition, histological analysis showed that anti-TNF treatment resulted in a significant reduction in joint erosion relative to control arthritic mice, as determined by the proportion of proximal-interphalangeal (PIP) joints showing erosive changes (Table I).

The long-term administration to man of anti-TNF mAbs containing epitopes of murine origin may be limited by the development of a neutralizing antibody response (Waldmann 1991). A TNF receptor-IgG fusion protein (of entirely human origin but containing neo-epitopes at the junction of IgG and TNF receptor) may, therefore, provide a less immunogenic alternative to mAbs, provided that the fusion protein can be shown to be effective *in vitro*. In one study, a human p75 TNF receptor-Fc $\gamma$  fusion protein was found to reduce the clinical severity of CIA, whether administered before or after the onset of arthritis (Wooley et al. 1993a). We were subsequently able to confirm the clinical efficacy of this form of treatment in established CIA using a human p55 TNF receptor-IgG fusion protein. In addition, we found by histological assessment that treatment with p55 TNF receptor-IgG had resulted in a significant reduction in joint erosion (Table II) (Williams et al. 1995).

Studies to assess the effect of blocking IL-1 have also been carried out in CIA. Thus, IL-1ra and anti-IL-1 $\beta$  mAb both suppressed CIA when treatment was started before disease onset (Wooley et al. 1993b, Geiger et al. 1993). Furthermore, treatment after disease onset with polyclonal anti-IL-1 $\alpha/\beta$ , or anti-IL-1 $\beta$  alone, profoundly suppressed both inflammation and cartilage destruction (Van den Berg et

al. 1994). It is clear, then, that the pathogenesis of CIA. However, in the case of arthritis driven by TNF, the role of TNF in synovial communication) which demonstrate that the use of anti-TNF is a logical therapeutic target for the inhibition of the pathway of IL-1 production.

The studies described above indicate that anti-TNF therapy is effective in reducing joint erosion. This treatment also indicates that the presence of excess TNF in the joints of patients with RA is different, with the use of anti-TNF antibody by several workers.

Experiments on anti-TNF have shown the relevance for the treatment of RA. It has been demonstrated that anti-CD4 treatment after the time of immunization was as effective as evidence to support the use of anti-TNF (Herzog et al. 1987). In the case of arthritis, it is effective when given after the onset of disease, indicating that was confirmed that treatment alone was ineffective in human RA has a degree of transient effect, though many patients have shown cell depletion (Burmester et al. 1994). At least one placebo-controlled study has shown a lack of clinical benefit, though a degree of concordance between the two studies and a relative lack of efficacy.

Having established the efficacy of anti-TNF therapy after disease onset, the next step was the administration of T cell-targeted therapy. It has been shown that anti-TNF has a significant impact on CIA. In the case of established CIA were given anti-CD4 (YTS191.1.2/YTA) plus anti-TNF. The results showed that both a sub-optimal dose of anti-TNF (Williams et al. 1994). In the case of TNF were observed both in the prevention of new limb joint erosion of cartilage

TABLE II

Treatment	Dose	PIP joints with erosions
Saline	—	15/15 (100%)
Control mAb	300 $\mu$ g	15/15 (100%)
Anti-TNF (TN3-19.12)	300 $\mu$ g	9/15 (60%) P<0.05
Saline	—	11/12 (92%)
IgG control	100 $\mu$ g	6/6 (100%)
TNF receptor-IgG (p55-sf2)	100 $\mu$ g	6/12 (50%) P<0.05

Reductions in joint erosion following treatment of established CIA with anti-TNF mAb or p55 TNF receptor-IgG fusion protein. Both TNF-neutralizing agents were given three times over a 10 day period. At the end of the treatment period, sagittal sections of the PIP joints of the middle digits were studied in a blinded fashion for the presence or absence of erosions (defined as demarcated defects in cartilage or bone filled with inflammatory tissue). Based on original data published in Williams et al. (1992) and Williams et al. (1995).

such studies in terms of GI anti-TNF monoclonal y period, starting immediately a reduction in clinical ulating levels of anti-type otological analysis showed on in joint erosion relative on of proximal-interphal-

nAbs containing epitopes f a neutralizing antibody n protein (of entirely hu- f IgG and TNF receptor) to mAbs, provided that . In one study, a human ce the clinical severity of f arthritis (Wooley et al. al efficacy of this form of rec -IgG fusion pro- that treatment with p55 on in joint erosion (Table

been carried out in CIA. hen treatment was started 993). Furthermore, treat- or anti-IL-1 $\beta$  alone, pro- truction (Van den Berg et

al. 1994). It is clear, therefore, that IL-1 contributes significantly to the pathology of CIA. However, in the TNF $\alpha$  transgenic mouse, a disease model in which the arthritis is driven by TNF $\alpha$ , anti-IL-1 therapy also ameliorates disease (Kolias, personal communication). These data are consistent with our observations in RA, which demonstrate that TNF $\alpha$  regulates the production of IL-1, and is therefore a logical therapeutic target, although in CIA, unlike in RA, a TNF $\alpha$ -independent pathway of IL-1 production may exist in addition.

The studies described above indicate that inflammatory cytokine-targeted therapy is effective in reducing the severity of CIA, and our work on anti-TNF treatment also indicates that the duration of the therapeutic effect is dependent on the presence of excess anti-TNF mAb in the circulation. However, results in patients are different, with the clinical benefit outlasting the 'therapeutic levels' of antibody by several weeks (unpublished data).

Experiments on animal models have provided further data that may be of relevance for the treatment of human disease. Early studies, for example, demonstrated that anti-CD4 mAb could block the induction of CIA if injected close to the time of immunization (Ranges et al. 1985), and this kind of study was used as evidence to support the initiation of clinical trials of anti-CD4 in human RA (Herzog et al. 1987). In CIA, however, anti-CD4 treatment was found to be ineffective when given *after* collagen immunization (Brahn & Trentham 1984), a finding that was confirmed in a subsequent study which showed that anti-CD4 treatment alone was ineffective in established CIA (Hom et al. 1988). Anti-CD4 treatment in human RA has been shown in a number of open clinical trials to provide a degree of transient clinical benefit to some patients (Herzog et al. 1989), although many patients fail to respond to therapy in spite of severe peripheral T-cell depletion (Burmester et al. 1992, Choy et al. 1992). Furthermore, the results of at least one placebo-controlled trial of anti-CD4 therapy in RA clearly showed a lack of clinical benefit (van der Lubbe et al. 1994) and there is, therefore, a degree of concordance between human RA and murine CIA with respect to the relative lack of efficacy of anti-CD4 treatment in established disease.

Having established the distinction in the efficacy of anti-TNF and anti-CD4 therapy after disease onset we carried out a study to determine whether a combination of T cell-targeted therapy and TNF-targeted therapy would have a more significant impact on CIA than either treatment alone. Thus, DBA/1 mice with established CIA were given intraperitoneal injections of either a depleting anti-CD4 (YTS191.1.2/YTA3.1.2) alone, anti-TNF (TN3-19.12) alone or anti-CD4 plus anti-TNF. The results showed that anti-CD4 mAb acted synergistically with both a sub-optimal dose (50  $\mu$ g) and an optimal dose (300  $\mu$ g) of anti-TNF mAb (Williams et al. 1994). The beneficial therapeutic effects of anti-CD4 and anti-TNF were observed both clinically, in the suppression of paw-swelling and in the prevention of new limb involvement and histologically, in the protection against joint erosion of cartilage and bone. Mice treated with anti-TNF alone developed

#### IP joints with erosions

15/15 (100%)
15/15 (100%)
9/15 (60%) P<0.05
11/12 (92%)
6/6 (100%)
6/12 (50%) P<0.05

CIA with anti-TNF mAb or gents were given three times al sections of the PIP joints sence or absence of erosions nflammatory tissue). Based ns et al. (1995).

a non-neutralizing antibody response to the anti-TNF mAb which was completely blocked by concurrent anti-CD4 treatment, a finding that may be important in terms of long-term treatment in man.

We were subsequently able to provide evidence of synergy between anti-CD4 and TNF receptor-IgG fusion protein (Williams et al. 1995). As in the previous experiment, it was found that concurrent anti-CD4 treatment almost completely blocked the anti-TNF receptor-IgG fusion protein response, which in this experiment resulted in increased circulating levels of the fusion protein. One of the possible mechanisms, therefore, to account for the synergy between anti-CD4 and TNF receptor-IgG is the prevention of a neutralizing antibody response leading to increased half-life of the fusion protein. However, other mechanisms of synergy also exist because synergy was observed between anti-CD4 and anti-TNF mAb in the absence of significant changes in levels of free anti-TNF in the serum (Williams et al. 1994).

In conclusion, these studies have shown that anti-TNF treatment leads to a degree of amelioration of CIA that is significantly increased when combined with anti-CD4. It is possible that combination therapy, targeted at the autoimmune response as well as the inflammatory cytokine network will provide the basis for the future treatment of RA.

#### EVIDENCE FOR ROLE ON TNF $\alpha$ FROM CLINICAL TRIALS

Having gathered *in vitro* and *in vivo* evidence of an important role for TNF $\alpha$ , the concept that neutralizing doses of monoclonal anti-TNF $\alpha$  antibody administered systemically might suppress RA was finally tested in patients by clinical trials. The aims of these trials was firstly to establish proof of principle, i.e., that it was possible to demonstrate clinical efficacy of an anti-TNF $\alpha$  chimeric monoclonal antibody (cA2) by employing validated clinical and laboratory parameters and showing a dose-response effect; secondly, to assess whether the magnitude and duration of benefit were sufficiently significant to herald a therapeutic advance; and thirdly, to investigate the safety and tolerability of the chimeric monoclonal antibody. To date, results of three clinical trials which have been completed on 93 patients have clearly demonstrated a substantial benefit in the short term in 80–90% of patients, following intravenous injection of cA2, with excellent tolerability and few side-effects.

The first study was designed primarily to test our hypothesis that TNF was of importance in the pathogenesis of RA. In this pilot study, we recruited 20 patients from our own hospital clinic and following referral from outside physicians. Each patient had refractory disease, in that they had a relatively long disease duration (median of 10.5 years), had either failed therapy or had reacted badly to several standard disease modifying antirheumatic drugs (DMARDs) and had continuing evidence of active synovitis. Although patients were permitted to continue on

stable doses of non-steroids, any disease-modifying drugs were withdrawn over a 4-week period.

The antibody used in this study (cA2) is a chimerized (human) antibody for human TNF $\alpha$  with a molecular weight of 120 kDa. Although this was the first clinical trial for this disease and the dose was based on the CIA model as a guide, patients received either 2 or 4 infusions of cA2 over 4 weeks. The total dose delivered was 10 mg/kg.

The patients were followed up for 6 months. Measures of disease activity were assessed by means of the Paulus criteria, which consists of six independent variables: morning stiffness, joint count, ESR, CRP, HAQ, and a composite of our data (Elliott et al. 1995). The most encouraging result was the most rapid changes in morning stiffness and gradual but still marked changes in joint count. Other clinical measures of disease activity also showed improvement, supported by significant

#### IL-6 and measures of disease activity

Time (days after treatment)	(n)
0 (entry)	*60
1	14
7	40
14	32
28	10
56	3

\* Median (interquartile range)  
\*\* Not done.

\*Paulus criteria: Significant improvement at least 20% improvement of morning stiffness; at least 2 grade improvement in joint count.

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#### CAL TRIALS

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stable doses of non-steroidal anti-inflammatory drugs and low dose corticosteroids, any disease-modifying drugs which the patients were taking at screening were withdrawn over a 4-week period, prior to entry.

The antibody used in the study and in subsequent trials was cA2 (Centocor, Pa, USA) a chimerized (human IgG<sub>1</sub>/mouse F<sub>2</sub>) monoclonal antibody with specificity for human TNF $\alpha$ , with maintenance of high affinity binding (Knight et al. 1993). Although this was the first occasion that such an antibody had been used in rheumatic disease and the dosage required was unclear, we had preliminary evidence from the CIA model as a guide to the dose range (Williams et al. 1992). We administered either 2 or 4 infusions of cA2 over a period of 2 weeks, each infusion lasting 2 hours. The total dose delivered in each treatment regime was the same (20mg/kg).

The patients were followed using a number of different clinical and laboratory measures of disease activity and the overall response to treatment was assessed by means of the Paulus criteria\*, a composite disease activity index incorporating six independent variables (Paulus et al. 1990) modified to accommodate the format of our data (Elliott et al. 1993). The results from this small pilot study were most encouraging. 19 of the 20 completed all scheduled infusions, with the one patient dropping out at the 2-week infusion point because of a concomitant illness (bronchitis). Patients showed marked improvement in all clinical measures, with the most rapid changes seen in the duration of morning stiffness and pain score and gradual but still marked improvements in the swollen and tender joint counts. Other clinical measures including the grip strength and the patient's assessment of response also showed significant improvement. The clinical improvements were supported by significant falls in laboratory measures of disease activity including

TABLE III  
*IL-6 and measures of the acute phase response after treatment with cA2*

Time (days after treatment)	Measure			
	IL-6 (pg/ml)	SAA (mg/ml)	CRP (mg/l)	ESR mm/hour
0 (entry)	*60 (42, 104)	245 (127, 513)	40 (28, 67)	55 (24, 77)
1	14 (1, 43)	190 (61, 415)	26 (18, 57)	**ND
7	40 (8, 60)	58 (26, 148)	5 (2, 13)	26 (19, 43)
14	32 (1, 51)	80 (38, 220)	6 (2, 29)	27 (15, 52)
28	10 (2, 48)	118 (44, 300)	11 (4, 32)	23 (16, 57)
56	3 (0, 14)	105 (54, 140)	6 (3, 25)	30 (21, 55)

\* Median (interquartile range) of up to 20 patients per point.

\*\* Not done.

\*Paulus criteria: Significant improvement in at least 4 of 6 variables, defined as:

- at least 20% improvement in continuous variables (tender and swollen joint scores, duration of morning stiffness, ESR).
- at least 2 grade improvement in patient's and observer's assessment of disease severity.

the acute phase proteins, serum amyloid-A (SAA), C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR; Table III). The response in each of these measures was rapid, with maximal improvement from day 7 after treatment. Significant falls were also seen in serum levels of IL-6, an important cytokine involved in the hepatic acute phase response. For many patients, serum IL-6 levels had normalized by day 1 after treatment and remained low for the duration of the study (Table III and data not shown). Serial changes in serum IL-6, SAA and CRP in a patient with a good response to cA2 are shown in Fig. 3 and demonstrate normalization of IL-6 levels in advance of the acute phase proteins. The data are consistent with the hypothesis that TNF blockade leads to inhibition of IL-6 synthesis, with subsequent down-regulation of hepatic acute phase protein synthesis. Other laboratory measures which demonstrate a trend towards improvement included the white blood cell and platelet counts and the hemoglobin.

Although these results were obtained in an open label, uncontrolled trial, the consistency and extent of the clinical improvements and the parallel changes in laboratory measures of the acute phase response suggested that the responses

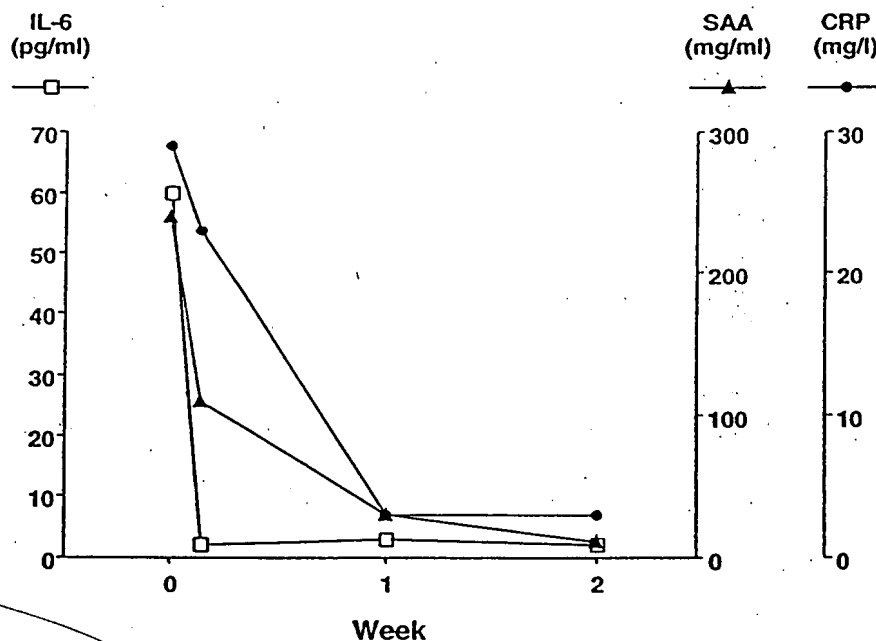


Figure 3. Changes in serum IL-6, SAA and CRP in a patient treated with cA2. The patient received a single infusion of 10 mg/kg cA2 at entry (week 0). IL-6 and SAA were measured by ELISA (Medgenix Diagnostics and Biosource International, respectively) and CRP were measured by rate nephelometry. Normal ranges: IL-6 <10 pg/ml; SAA <10 mg/ml; CRP <10 mg/ml.

were likely to be due to the importance of TNF in this disease, we compared the use of TNF inhibitors. Many of the designatory disease, the requirement for stable doses of TNF inhibitors in this study. Patients were given 10 mg/kg and were

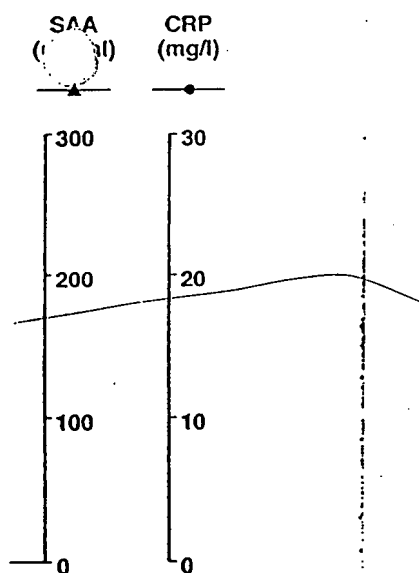
The predetermined week 4 of a response to treatment. There were substantial differences between the 24 placebo recipients and the 24 patients treated with high-dose TNF inhibitors. Improvements in individual changes were seen in the study. In particular, the proportion of morning stiffness

20% Response

50% Response

Figure 4. Responses at week 4 to cA2 or placebo. Patients' responses were calculated as the percentage of values represent significance

ve protein (CRP) and response in each of day 7 after treatment. Important cytokine levels, serum IL-6 levels for the duration of serum IL-6, SAA and in Fig. 3 and demonstrate phase proteins. The leads to inhibition of acute phase protein a trend towards improvement and the hemoglobin. In a controlled trial, the parallel changes in and that the responses



with cA2. The patient and SAA were measured actively) and CRP were  $A < 10$  mg/ml; CRP  $< 10$

were likely to be due to a true therapeutic effect. As the definitive test of the importance of TNF in the pathogenesis of RA and of the clinical efficacy of cA2 in this disease, we devised a multi-center, randomized, double-blind study which compared the use of cA2 to placebo (human serum albumin) (Elliott et al. 1994a). Many of the design features, including the requirement for longstanding, refractory disease, the requirement for withdrawal from DMARD therapy and the need for stable doses of other concomitant medications were maintained into the 2nd study. Patients were given a single infusion of placebo or cA2 (at a dose of 1 or 10 mg/kg) and were followed by blinded observers for response.

The predetermined, primary endpoint of the study was the achievement at week 4 of a response according to the Paulus (20%) criterion. Using this measure there were substantial and clear differences between the 3 groups, with only 2 of the 24 placebo recipients (8%) responding compared with 19 of 24 patients (79%) treated with high-dose cA2. The responses of the low-dose cA2 recipients were intermediate, demonstrating a dose-response relationship (Fig. 4). If the threshold of improvement was raised to a 50% improvement in Paulus index at a 4-week end-point, then 58% of patients treated with the higher dose of cA2 gained benefits from treatment (Fig. 4). Although considered supportive in nature, the improvements in individual disease activity measures were also of interest and similar changes were seen in the cA2 treated groups to those seen in the open label study. In particular, the tender and swollen joint counts, the pain score, the duration of morning stiffness, the fatigue score, the grip strength and the patients'

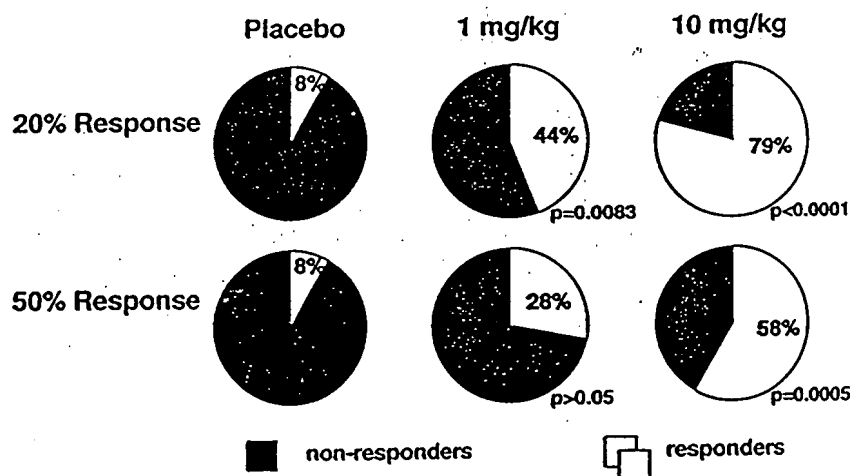


Figure 4. Responses at week 4 in patients treated with high (10 mg/kg) or low (1 mg/kg) dose cA2 or placebo. Patients received a single infusion of cA2 or placebo at entry (week 0). Responses were calculated according to Paulus 20% or 50% criteria, as described in the text. p values represent significance of changes compared with placebo by Fisher's exact test.

and observers' assessments of disease severity all showed highly significant improvements. These changes were matched by improvements in the ESR and CRP, particularly in the high-dose cA2 group. As in the open label study, the infusions were well tolerated and though a variety of adverse events were seen, the overall safety profile of cA2 was good, and the adverse events were not greater at high doses of cA2 than placebo.

One element of particular interest in this study was the improvement in the hemoglobin concentration in patients treated with high-dose cA2, compared with a fall in placebo-treated patients. Although these changes were small in magnitude, they occurred over a period of only 4 weeks in the setting of significant venesection for safety monitoring, and the difference between the changes in placebo and high-dose cA2 groups was highly significant. The anemia of chronic disease seen in RA is multifactorial in origin but recent evidence suggests that TNF $\alpha$  has a direct effect in the bone marrow in suppressing erythropoiesis (Roodman 1987, Johnson et al. 1989). While the improvements in hemoglobin in cA2 treated patients might be simply a reflection of improvement of overall disease state, a more interesting hypothesis is that cA2 is directly interfering with TNF $\alpha$  mediated suppression of erythropoiesis in the bone marrow.

The results of this study confirm that cA2 is a highly effective agent in the short-term suppression of RA and strongly support the hypothesis that TNF is a significant contributor to the inflammatory state in this disease. The findings do not however directly address the question of the relative importance of TNF $\alpha$  and IL-1 in disease pathogenesis. No full-length reports are yet available describing the use of specific IL-1 blockers in RA, but preliminary data presented in abstract form suggest some therapeutic efficacy for both IL-1ra (Lebsack et al. 1993) and a soluble receptor for IL-1 (Drevlow et al. 1993).

The data presented so far suggests that cA2 may find a role as a remission inducing agent in RA, or in the control of acute disease flares. A more ambitious goal for a new therapeutic agent, however, would be long-term disease suppression and in particular the achievement of disease modification. Although many of the currently available drugs in the treatment of rheumatic disease are termed 'disease modifying', the evidence that they do in fact alter outcomes is at best arguable (Brooks 1993). TNF $\alpha$  has major effects on cartilage metabolism, including the inhibition of collagen synthesis and the stimulation of collagenase production by fibroblasts and synovial cells, together with both direct and indirect effects on proteoglycan metabolism (reviewed by Vassalli 1992). It might be expected, therefore, that long-term TNF blockade would favorably influence cartilage metabolism and reduce tissue damage.

Experience with cA2 suggests that, although it is a reliable disease suppressing agent, the responses are transient, with patients showing disease relapse after a period of months after the antibody disappears from the circulation. In our first experience in the repeated use of cA2, we administered cycles of therapy to a small

number of patients originally completed between 2 and 4 upon evidence of disease (Elliott et al. 1994b), with maintenance of response duration. Of particular interest is the immunogenicity, since the murine responses upon anti-murine response in patients re-treated with

The clinical significance are as yet unclear, but in long-term treatment either blocking cA2 binding sites or the major therapeutic agent engineered thereapeutic tially immunogenic (Is) adopted to circumvent antibodies to CD4 to block administered anti-TNF models.

Preliminary reports made recently. In one, to patients with active disease, improvements were seen including the tender joint count study employed a fusion protein coupled to a humanizing study incorporating cutaneous administration of significant improvements were seen in joint counts, the patient morning stiffness, together and further validate the results of trials in progress were summarized in Table IV.

#### MECHANISM OF

The 'pleiotropy' of TNF types, driving multiple



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pleted between 2 and 4 complete treatment cycles, with each cycle administered  
upon evidence of disease relapse. The findings have been reported in full recently  
(Elliott et al. 1994b), with repeated responses after each cycle administered, and  
maintenance of response magnitude, but a trend (non-significant) to shortening re-  
sponse duration. Of particular interest in this study was the tissue of immuno-  
genicity, since the murine variable region of cA2 might be expected to stimulate anti-  
murine responses upon repeated dosing. Although only 1 patient had developed an  
anti-murine response in the original open label trial (unpublished data), 50% of the  
patients re-treated with cA2 developed such responses, mostly at a low titer.

The clinical significance of these immunological responses to injected antibody  
are as yet unclear, but it is possible that they could limit therapeutic efficacy with  
long-term treatment either through accelerated clearance of injected cA2, or by  
blocking cA2 binding sites to TNF $\alpha$ . Overcoming these responses is likely to repre-  
sent the major therapeutic challenge for the repeated use of cA2, or indeed any other  
engineered therapeutic antibodies, since even 'humanized' antibodies are poten-  
tially immunogenic (Isaacs et al. 1992). While a number of approaches could be  
adopted to circumvent the development of such responses, administration of anti-  
bodies to CD4 to block the development of antiglobulin responses to concurrently  
administered anti-TNF antibody has been discussed in the previous section on ani-  
mal models.

Preliminary reports from 2 other studies targeting TNF $\alpha$  in RA have been  
made recently. In one, a humanized murine antibody (CDP571) was administered  
to patients with active rheumatoid arthritis in a placebo controlled, dose escala-  
tion study (Rankin et al. 1994). The antibody was well tolerated and significant  
improvements were seen in a number of individual disease activity measures, in-  
cluding the tender joint count, the pain score and the ESR. A second reported  
study employed a fusion protein, comprising recombinant human p75 TNF recep-  
tor coupled to a human IgG1 Fc framework (Moreland et al. 1994). This dose-  
ranging study incorporated intravenous loading at entry, with twice-weekly sub-  
cutaneous administration of the fusion protein for maintenance therapy. Signifi-  
cant improvements were seen compared to baseline for the tender and swollen  
joint counts, the patient and physician global assessments and the duration of  
morning stiffness, together with the ESR. These data lend support to our findings  
and further validate the clinical efficacy of TNF $\alpha$  blockade. The current status  
of trials in progress with anti-cytokines mostly published in abstract form, is  
summarized in Table IV.

#### MECHANISM OF ACTION OF ANTI-TNF $\alpha$ ANTIBODY THERAPY

The 'pleiotropy' of TNF action, jargon for its multitude of effects on various cell  
types, driving multiple biological processes, is the likely reason for the marked

TABLE IV  
Anti-cytokines in clinical trials in RA

Target	Therapy	Results
TNF $\alpha$	● Monoclonal anti-TNF $\alpha$ antibody:	
	– Chimeric (cA2) (Centocor)*	*RCT: beneficial
	– humanised (CDP571) (CellTech)	Dose escalation: beneficial
	● Soluble TNF-R-Ig fusion proteins:	
	– TNFR:Fc (p55) (Roche)	In trials: beneficial
	– TNFR:Fc (p75) (Immunex)	Dose ranging: beneficial
IL-1	● IL-1RA (Synergen)	Dose ranging: beneficial
	● Soluble IL-1R (Immunex)	RCT: trend to beneficial
IL-6	● Monoclonal anti-IL-6 antibody	
	– murine (BE-8)	Open label: beneficial

\*RCT=randomized placebo-controlled trial.

\*=published as full papers, rest are published in abstract form.

effects of anti-TNF therapy *in vivo*. However, it remains to be established which of these many effects of TNF, inhibited by anti-TNF, are of central importance in the therapeutic effect. Our current working hypothesis, based on the results so far that the cA2 antibody has two major effects. It interrupts the cytokine cascade *in vivo*, just as it does in the *in vitro* rheumatoid synovial cultures. The best clinical evidence for interruption of the cytokine cascade *in vivo* is the rapid and marked reduction in the CRP, the rise of which in RA reflects the cytokine activation of the hepatocytes, probably chiefly by IL-6, but possibly involving any of the cytokines whose signals are mediated by gp130 (e.g. LIF, IL-11). Supporting evidence for widespread cytokine blockade has been obtained by the fall in serum IL-6 levels by ELISA (Fig. 3). Cytokine blockade and diminution of events downstream is probably of major importance in the rapidity of onset of clinical benefit, and in the reduction in local inflammatory features, such as morning stiffness, pain and joint swelling.

However, an important feature of cA2 therapy is the duration of clinical benefit, much beyond the fall of serum levels of anti-TNF to levels not capable of neutralizing TNF $\alpha$  bioactivity within the RA joints. A number of lines of evidence have converged to suggest the hypothesis that cA2 has a major effect on the recruitment and trafficking of blood cells to the joint. Endothelium in RA synovium has been reported to express increased levels of adhesion molecules for leukocytes, such as E-selectin, VCAM-1 and ICAM-1 (Koch et al. 1991, Fischer et al. 1993, Johnson et al. 1993, Cronstein 1994, Szekanecz et al. 1994). Since blood cells, neutrophils, but especially macrophages and T cells, are needed for maintaining the disease process and do not proliferate much locally, interruption of the influx of cells may be of major significance in the duration and depth of

Figure 5. Reduction of anti-TNF $\alpha$  monoclonal 14 days after cA2 infusion the degree of inflammation by scoring the thickness of aggregates (Firestein et al. 1994) in the two sections of each

benefit. Moreover, it destructive process it reducing the clinical existing drugs.

The clues we have synovial histology, w also been studied, ari vation, soluble E-sele

Synovial biopsies t patients in our first t were taken from the s from each block of bi lining layer, cellular i parameters were used brane. We found the : 1. In the 3 patients w from a mean value of

**Results**

CT: beneficial  
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 dose ranging: beneficial  
 CT: trend to beneficial

open label: beneficial

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is to establish which are of central importance is, based on the results so disrupts the cytokine cascade cultures. The best clinical is the rapid and marked the cytokine activation of involving any of the cyto- (IL-1). Supporting evidence by the fall in serum IL-6 initiation of events down- of onset of clinical benefit, such as morning stiffness,

duration of clinical bene- to levels not capable of number of lines of evi- cA2 has a major effect on int. Endothelium in RA of adhesion molecules for Koch et al. 1991, Fischer Zencz et al. 1994). Since d T cells, are needed for much locally, interruption e duration and depth of

### Inflammatory Scores

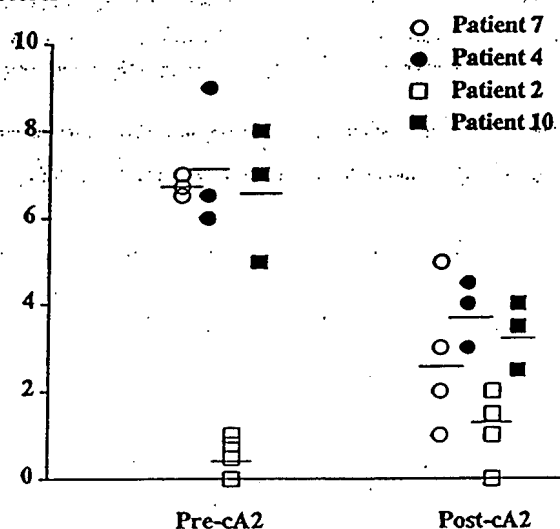


Figure 5. Reduction of inflammation in the synovial membrane in RA patients treated with anti-TNF $\alpha$  monoclonal antibody (cA2). Multiple synovial biopsies were taken before and 14 days after cA2 infusion from 4 patients in the 'open-label' clinical trial and analyzed for the degree of inflammation. Two distant sections from each biopsied sample were graded by scoring the thickness of the lining layer, cellular infiltration and the number of lymphoid aggregates (Firestein et al. 1991). Each point in the figure represents the average value of the two sections of each biopsy.

benefit. Moreover, it suggests that blocking TNF long-term could interrupt the destructive process itself, which needs cell influx to sustain it, rather than merely reducing the clinical features such as pain and swelling more effectively than existing drugs.

The clues we have obtained to support the role of trafficking have come from synovial histology, where the adhesion molecules on synovial endothelium have also been studied, and from analysis of the serum marker of endothelial activation, soluble E-selectin.

Synovial biopsies before and 14 days after cA2 infusion were available from 4 patients in our first 'open-label' clinical trial. In each patient, at least 3 biopsies were taken from the same knee joint before and after therapy. Two distant sections from each block of biopsied tissue were stained and examined for thickness of the lining layer, cellular infiltration and the number of lymphoid aggregates. These parameters were used to score the degree of inflammation of the synovial membrane. We found the score to be high in 3 patients and only minimally raised in 1. In the 3 patients with the highest inflammatory score, there was a reduction from a mean value of 6.8 to 3.5 following cA2 therapy (Fig. 5). Other histological

features were assessed. The synovial lining layer is relatively uniform in individual joints, in contrast to the sublining layer which shows regional variability. The lining layer decreased from 6-8 cells thick to 3-4 cells thick following therapy. Lymphoid aggregates are patchy in distribution; nevertheless these were scored and showed a reduction from 5 to 3.5 per low-power field. Since neovascularization is a prominent feature of the RA synovium, the number of blood vessels (defined by staining with antibody to von Willebrand Factor and a specific anti-endothelial antibody EN4) (Ruiter et al. 1989) was compared. There was no significant change in the number of vessels in pre- and post-treatment specimens.

Since trafficking of leukocytes to the joint is an important process in the pathogenesis of rheumatoid arthritis and augmented expression of endothelial adhesion molecules is involved in this process, we monitored the expression of ICAM-1, VCAM-1 and E-selectin on vascular endothelium in the biopsies. ICAM-1 and VCAM-1 were detected on most vascular endothelial cells, as well as many of the lining layer cells. This is expected, based on the expression of these molecules on antigen-presenting cells as well as endothelial cells. There was a tendency for VCAM-1 staining on endothelial cells, but not on lining cells, to be reduced post-treatment, but no changes in ICAM-1 expression on endothelium were noted. Antibody to E-selectin exclusively stained 3-28% of vascular endothelial cells on capillaries and venules. The expression of E-selectin was significantly reduced in the post-treatment samples (Fig. 6).

Recently it has become apparent that many adhesion molecules, like cytokine receptors, also exist in the serum in a soluble form, to complement the cell surface form. Their function in the serum is not clearly documented, and unlike sTNF-R, which was discovered as a urinary TNF inhibitor, it is not yet known whether they are competitive inhibitors. However, it is known that these molecules are derived from proteolytic cleavage of the surface form, as no alternatively spliced mRNA has been found, at least for ICAM-1 and E-selectin (Gearing & Newman 1993). Release of soluble ICAM-1 and E-selectin has been found to correlate with expression of these molecules on the surface of cytokine-activated cultured endothelial cells (Leeuwenberg et al. 1992, Pigott et al. 1992). Elevated serum levels of ICAM-1, VCAM-1 and E-selectin have been observed in RA (Cush et al. 1993, Koch et al. 1993, Wellicome et al. 1993), although only ICAM-1 and VCAM-1 appear to correlate with disease severity (ESR) (Aoki et al. 1993, Mason et al. 1993).

In order to further assess the significance of the reduction in E-selectin observed on synovial blood vessels, levels of soluble E-selectin were assayed in serial serum samples, and were found to be diminished by cA2 therapy in 19/20 patients. The earliest observed decrease was at day 7, although serum levels appeared to be attenuated even at 4 and 6 weeks (Fig. 7). In some patients levels returned to baseline by day 56 (in 5/20 levels were rising by weeks 6 to 8); however, in most patients levels were still below pre-treatment levels at that time. The initial levels

E-selectin-stained  
blood vessels

Figure 6. Decrease of E-selectin in RA patients treated with BBA1. The expression of E-selectin on blood vessels (as stated in Fig. 1) were significantly reduced in the post-treatment samples in the same 3 patients.

in the 20 patients varied (range 0-28%). In 1 patient, in whom the initial level was 28%, the level decreased to 3%.

Levels of soluble E-selectin in serum were also assayed. There was an apparent decrease in soluble E-selectin levels in 19/20 patients. The initial level was 351.7 ng/ml. The difference was not statistically significant. Four weeks post-treatment, the level was 100 ng/ml, a significant decrease from pre-infusion value (p < 0.05). Soluble VCAM-1 at any time point was not assayed.

Rheumatoid arthritis therapies are of limited efficacy. The initial approach to u

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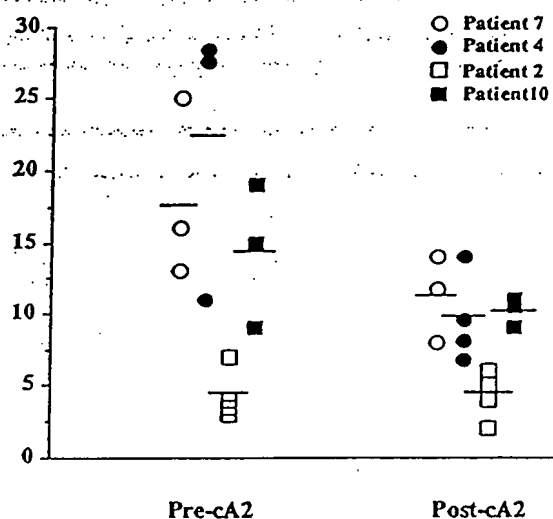


Figure 6. Decrease of E-selectin expression on endothelial cells in the synovial membrane in RA patients treated with anti-TNF $\alpha$  monoclonal antibody (cA2). The synovial biopsies (as stated in Fig. 1) were stained with a mouse monoclonal anti-human E-selectin antibody (BBA1). The expression of E-selectin on endothelial cells was reduced in the post-treatment samples in the same 3 patients that showed reduced inflammatory scores.

in the 20 patients varied considerably from 41–224 ng/ml (mean normal 47.6 ng/ml). In 1 patient, in whom no diminution of soluble E-selectin levels was observed, the initial levels were in the normal range (<50ng/ml).

Levels of soluble ICAM-1 were assayed in only 3 patients, and also exhibited an apparent decrease following cA2 therapy. Mean serum ICAM-1 levels decreased from 351.7 ng/ml (pre-infusion) to 262.3 ng/ml by day 7, although this difference was not statistically significant due to the low number of patients tested. Four weeks post-infusion the levels of ICAM-1 appeared to be returning to pre-infusion values. Finally, sera from a total of 10 patients were assayed for soluble VCAM-1. There was no significant difference detectable in levels of soluble VCAM-1 at any time after cA2 infusion (Fig. 7).

#### SUMMARY

Rheumatoid arthritis is a common cause of chronic disability for which current therapies are of limited value in controlling the disease process and outcome. Our initial approach to understanding the pathogenesis of RA and defining a novel

## Serum adhesion molecule levels after anti-TNF infusion

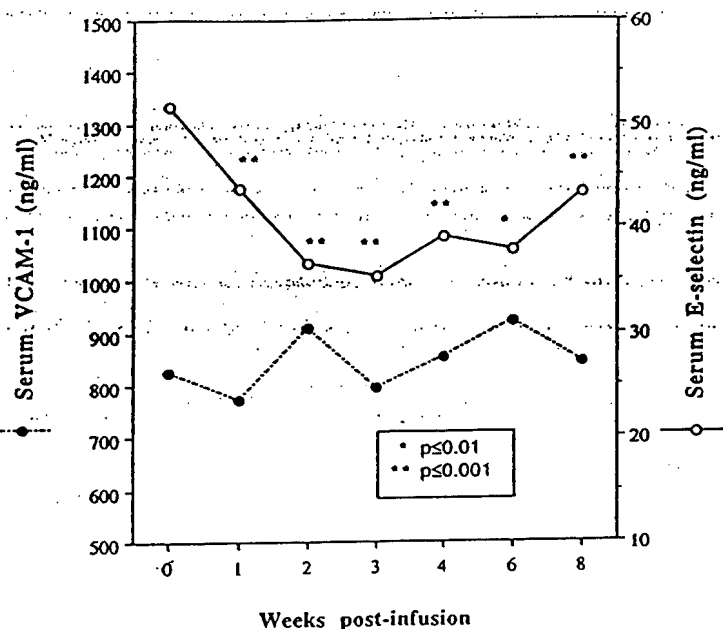


Figure 7. Serum adhesion molecules after anti-TNF infusion. Circulating levels of serum E-selectin (—○—) VCAM-1 (—●—) were measured by ELISA (British Biotechnology Products Ltd, UK) after infusion of cA2. Values are means of 20 and 10 patients respectively. Significant differences versus pre-infusion values were determined by Wilcoxon signed rank test (\*)  $p < 0.01$ , (\*\*)  $p < 0.001$ .

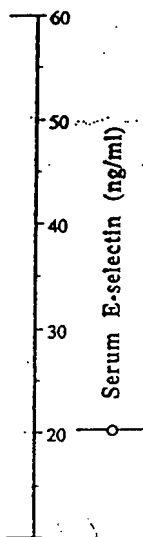
therapeutic target was to investigate the role of cytokines by blocking their action with antibodies on cultured synovial-derived mononuclear cells *in vitro*. These investigations suggested that neutralization of TNF $\alpha$  with antibodies significantly inhibited the generation of other pro-inflammatory cytokines also over-produced, such as, IL-1, GM-CSF, IL-6 and IL-8. The implication that blockade of a single cytokine, TNF $\alpha$  might have far-reaching effects on multiple cytokines and thereby exert significant anti-inflammatory and protective effects on cartilage and bone of joints, was tested in arthritic DBA/1 mice immunized with collagen II. Impressive amelioration of joint swelling and joint erosions in this model encouraged clinical trials with a monoclonal anti-TNF $\alpha$  antibody. The cA2 chimeric anti-TNF $\alpha$  high-affinity antibody was initially tested in an open-label study at a dose of 20 mg/kg on 20 patients, with substantial and universal benefit. Subsequently, a randomized placebo-controlled double-blind trial was performed on 73 patients comparing a

single intravenous infusion of doses of cA2. Using 8% of patients received cA2 and 79% received administered to 7 patients with each cycle indicated in the pathogenesis of anti-TNF $\alpha$  antibody in long-term control of RA. Idiopathic responses in the induction of response. In addition, anti-CD4 is observed indicating one way in

The Kennedy Institute funded research institution for preparation of the

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 Buchan, G., Barrett, J.

## TNF infusion



single intravenous injection of placebo (0.1% human serum albumin) with two doses of cA2. Using a composite disease activity index, at 4 weeks post infusion, 8% of patients receiving placebo improved compared with 44% receiving 1mg/kg cA2 and 79% receiving 10 mg/kg. Between 2 to 4 repeated cycles of cA2 were administered to 7 patients and all patients showed improvement of a similar magnitude with each cycle. These data support our proposition that TNF $\alpha$  is implicated in the pathogenesis of RA, and is thus a key therapeutic target. Monoclonal anti-TNF $\alpha$  antibodies control disease flares and are candidate agents for longer-term control of RA, although repeated therapy with cA2 is associated with anti-idiotypic responses in 50% of patients and a trend toward shortening of the duration of response. In the DBA/1 arthritic mice, synergy of action of anti-TNF and anti-CD4 is observed together with suppression of an anti-globulin response, indicating one way in which benefit might be augmented in the future.

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